Pesq. Vet. Bras. 31(5):425-429, maio 2011

Anticoccidial activity of hydrochloric acid (HCl) against *Eimeria tenella* in broiler chickens[1]

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ABSTRACT.- Abbas R.Z., Manzoor Z., Munawar S.H., Iqbal Z., Khan M.N., Saleemi M.K., Zia M.A. & Yousaf A. 2011. **Anticoccidial activity of hydrochloric acid (HCl) against *Eimeria tenella* in broiler chickens.** *Pesquisa Veterinária Brasileira 31(5):425-429.* Department of Parasitology, University of Agriculture, Faislabad - 38040, Pakistan. E-mail: raouaf@hotmail.com

The present study was planned to evaluate the anticoccidial activity of the different concentrations of the HCl against *Eimeria tenella* infection in broiler chickens in comparison with the amprolium anticoccidial. For this purpose, a total of 198 chicks were placed 11 per pen with three pens per treatment. The different concentrations of HCl (1000ppm, 2000ppm and 3000ppm) and amproilum (at the dose rate of 125ppm) were given to the experimental groups in drinking water from 10 to 19th days of age. One group was kept as infected non medicated control and one as non infected non medicated control. At the 12th day of age, all the groups were inoculated orally with 75,000 sporulated oocysts except non infected non medicated control. Anticoccidial activity was evaluated on the basis of performance (weight gain, feed conversion ratio) and pathogenic (oocyst score, lesion score and mortality %age) parameters. Among HCl medicated groups, the maximum anticoccidial effect was seen in the group medicated with 1000ppm HCl followed by 2000ppm and 3000ppm HCl medicated groups. Amprolium and 1000ppm HCl were almost equivalent in suppressing the negative performance and pathogenic effects associated with coccidiosis (*Eimeria tenella*) challenge. In summary, the lower doses of HCl have the potential to be used as alternative to chemotherapeutic drugs for *Eimeria tenella* control. It is therefore suggested that further studies should be carried out to determine the possible minimum safe levels of HCl with least toxic effects to be used as anticoccidial.

INDEX TERMS: Coccidiosis, *Eimeria tenella*, HCl, anticoccidial.

INTRODUCTION

Commercial poultry farming in Pakistan is expanding day by day. Poultry meat contributes about 19% of the total meat production in the country (Ahmad et al. 2010, Ghafoor et al. 2010) and is one of the best available sources for the production of high biological value animal protein. However, this sector is still confronted with many problems like coccidiosis which are hindering its progress (Saima et al. 2010).

Avian coccidiosis is an intestinal disease caused by obligatory protozoan parasites belonging to genus *Eimeria*. *Eimeria tenella* primarily invades and resides in the lining of the cecum of exposed chickens (Allen 1999, Williams 1999, Laurent et al. 2001) and resulting in extensive destruction of the cecal epithelium, hemorrhagic feces, reduction in body weight gain, and decrease in feed efficiency and eventually mortality. Thus far, chemoprophylaxis and anticoccidial feed additives have controlled the disease but have been complicated by the emergence of drug resistance (Abbas et al. 2008) and their toxic effects on the animal health (Nogueira et al. 2009). Furthermore, drug or antibiotic residue in the poultry products is potentially annoyance to consumer.

Therefore, alternative strategies are being sought for more effective and safer control of coccidiosis in broilers (Dalloul

[1] Received on November 3, 2010.
Accepted for publication on January 4, 2011.

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& Lillehoj 2006, Abbas et al. 2010). Acids such as fumaric, propionic, sorbic, acetic, and tartaric have also been reported for positive influence on feed conversion ratio and growth performance (Vogt et al. 1981, 1982).

In Pakistan, HCl is being used extensively as anticoccidial agent in the local poultry industry. According to poultry producers, HCl has been found effective against avian coccidiosis, but there is no scientific report available regarding its anticoccidial activity. Therefore, this study has been planned to evaluate the possible anticoccidial effect of HCl, if any, employing standard parasitological procedure.

MATERIALS AND METHODS

Experimental birds, feed and management. One hundred and ninety eight (1-day-old) broiler chicks (Hubbard Al-Noor Chicks, Pvt) were purchased from local hatchery. Chicks were reared under standard management practices. All the chicks were kept on broiler starter ration up to 2 weeks of age and then fed a broiler finisher. The feed and water were provided *ad libitum*. The temperature was maintained at 29.5-32.5ºC during the first week of age and was reduced by 3ºC on weekly basis. Lighting was provided for 24 hours through out the experimental period. All the birds were vaccinated for New Castle disease on 5th day, for Infectious Bursal disease on 14th day, and for Hydro pericardium syndrome on 18th day of age.

Parasite. Coccidial oocysts were obtained from the caeca of infected chickens and were propagated in broiler chickens by giving oral infection. After obtaining sufficient amount of oocysts, they were sporulated by placing in 2.5% $K_2Cr_2O_7$ in the presence of suitable humidity and temperature. Sporulated oocysts were cleaned and counted by the McMaster technique (MAFF 1986). The required concentration of the sporulated oocysts (75,000/ml) was maintained with phosphate buffered saline.

Study design. The chicks (n=198) were placed 11 per pen with three pens per treatment. Treatments were randomized within blocks. Treatments were as follows:

1000ppm HCl medicated group, 2000ppm HCl medicated group, 3000ppm HCl medicated group (in Pakistan, the poultry farmers use 2000ppm HCl in drinking water to prevent the coccicidosis, therefore, in the present study; we used one concentration up (3000ppm) and one down (1000ppm) which is the normal approach for the scientific validation of the ethno veterinary medicines). Amprolium medicated group (amprolium was used at the recommended dose rate of 125ppm) infected non medicated control; non infected non medicated control. All the groups were inoculated orally with 75,000 sporulated oocysts at the 12th day of age except non infected non medicated control. Amprolium (at the dose rate of 125ppm) and different concentrations of HCl were given in water from 10 to19th days of age.

Evaluation of anticoccidial effects. Five chicks from each group were weighed on day of inoculation (12th day of age) and then reweighed on 7th day post inoculation (19th day of age).

Feed conversion was calculated as the grams of feed consumed to produce one gram of live weight. Statistical analysis of FCR was not possible because of group feeding of birds.

Five chicks from each group were sacrificed for post mortem examination at 7th day of post inoculation (19th day of age). Caecal lesions were scored by the lesion scoring technique described by Johnson & Reid (1970).

An oocyst index (0 to 5) was determined by microscopical examination of scrapings from the caeca of chicks sacrificed for lesion scoring at 7th day of post inoculation (Hilbrich 1978).

Caecal contents of three chicks from each group were removed as soon as possible for the pH measurements on days 3, 5 and 7 post inoculation following procedures outlined by Ruff et al. (1974).

Statistical analysis. Data obtained on various parameters were analyzed by analysis of variance, and the mean values were compared by Tukey test. The differences among group means were considered significant at $P<0.05$.

RESULTS

The results of weight gain (Table 1) showed that the body weight gains in all the medicated groups except 3000ppm HCl medicated group were significantly ($P<0.05$) higher than infected non medicated control. Among HCl medicated groups, the maximum weight gain was shown by the group medicated with 1000ppm HCl followed by the group medicated with 2000ppm HCl.

The results of FCR (Table 1) revealed that the FCR values of HCl medicated groups were numerically lower compared with infected non medicated group, although a statistical comparison could not be made due to group feeding. HCl medicated groups showed numerically lower FCR compared with amprolium medicated group, except for 3000ppm HCl medicated group. Among HCl medicated groups, the lowest FCR was observed in the group medicated with 1000ppm HCl followed by the groups medicated with 2000ppm HCl and 3000ppm HCl.

The results of the lesion scores are also indicated in Table 1. The uninfected control group showed zero score. All the groups medicated with HCl and amprolium showed significantly ($P<0.05$) lower lesion scores than infected non medicated group except 3000ppmHCl medicated group. Among all medicated groups, the lowest lesion scores were shown by the group medicated with amprolium, although statistically non significantly ($P>0.05$) in comparison with 1000ppm HCl and 2000ppm HCl medicated groups.

The results of the oocyst scores (Table 1) revealed a pattern relatively similar to that of lesion scores among different groups. The oocyst scores were lower ($P<0.05$) in medicated groups (except 3000ppmHCl medicated group) compared with infected non medicated group. Among medicated groups the maximum reduction in oocyst scores were seen in the amprolium medicated group followed by 1000ppm HCl, although statistically non significant ($P>0.05$) and 2000ppm HCl medicated groups respectively. The results revealed that the HCl (1000ppm and 2000ppm) medicated groups have anticoccidial activity in terms of oocyst score.

The percent mortality (Table 1) was higher in the infected non-medicated control group compared with medicated groups. Among medicated groups, the mortality was numerically lower in amprolium medicated group followed by groups medicated with 1000ppm, 2000ppm and 3000ppm HCl respectively.

The results on the pH of cecal contents in the different experimental groups are shown in (Table 2). On all days (3,

Table 1. Comparative values of the mean (and SEM) weight gain, feed conversion ratio, lesion score, oocyst index, and mortality percentage

Treatments	Weight gain	Feed conversion ratio(g/g)*	Lesion score	Oocyst score	Mortality % age
HCl (1000ppm)	283[a]	1.13	2.5[b]	1.5[c]	7.5
HCl (2000ppm)	272[a]	1.26	2.9[b]	2.7[b]	12.6
HCl (3000ppm)	259[b]	1.37	3.4[a]	3.6[ab]	20.4
Amprolium (125ppm)	285[a]	1.34	2.3[b]	1.3[c]	5.2
Infected non medicated	257[b]	1.43	3.6[a]	4.6[a]	23.4
Non infected non medicated	287[a]	1.32	-	-	-
S.E.M.	17.3	-	0.24	0.41	-

[a-c] Means shearing similar superscripts within a column do not differ (P<0.05).
* Statistical analysis was not possible because of group feeding of chicks.

Table 2. Effect of HCl treatment on mean (n = 3) pH of caecal contents (on 3, 5 and 7th days post inoculation) in broiler chickens

Treatments	3rd day	5thday	7thday
HCl (1000ppm)	6.22[c]	6.18[c]	6.25[c]
HCl (2000ppm)	6.14[c]	5.89[c]	5.98[c]
HCl (3000ppm)	5.83[c]	5.81[c]	6.12[c]
Amprolium (125ppm)	6.53[b]	6.75[b]	6.82[b]
Infected non medicated	6.96[a]	7.23[a]	7.42[a]
Non infected non medicated	6.52[b]	6.64[b]	6.92[b]
S.E.M.	0.002	0.005	0.004

[a-c] Means shearing similar superscripts within a column do not differ (P<0.05).

5 and 7 post inoculation), a significant (P<0.05) difference was observed among intestinal pH of the HCl medicated groups, amprolim medicated group and non infected non medicated control, and infected non medicated control. The maximum pH was observed in the infected non medicated control followed by amprolium medicated group and HCl medicated groups. There was no significant (P<0.05) difference of pH between amprolium medicated group and non infected non medicated control.

DISCUSSION AND CONCLUSIONS

In the present study, lower doses of HCl administered in drinking water has shown the anticoccidial effects against *Eimeria tenella* in terms of improved weight gains, better FCR, lower oocyst and lesion scores. The coccidiosis can lead to enormous economic losses in the poultry industry due to the intestinal lesions caused by the *Eimeria* species and subsequent malabsorption of nutrients. The performance improvements observed while using HCl (particularly 1000ppm) could overcome these losses.

Hydrochloric acid is a solution of hydrogen chloride in water. It is found naturally in gastric acid. Historically called muriatic acid or spirits of salt, hydrochloric acid was produced from vitrio and common salt. Hydrochloric acid is a monoprotic acid, which means it can dissociate (ionize) only once to give up one H^+ ion (a single proton). In aqueous hydrochloric acid, the H^+ joins a water molecule to form a hydronium ion, H_3O^+. The other ion formed is Cl^-, the chloride ion. Hydrochloric acid is a strong acid, since it is essentially completely dissociated in water (Perry et al. 1984, Lide 2000).

Very limited research exists on the effects of acids on poultry health particularly during coccidiosis. However, few reports (Chaveerach et al. 2004, Van Immerseel et al. 2004) are available regarding the antimicrobial effects of acids. These acids showed promise in altering bacterial activities and cecal environment in chicken.

The results on the improved body weight gains and FCR shown in this work are in line with a number of previous reports (Vogt et al. 1981, Patten & Waldroup 1988, Owings et al. 1990, Skinner et al. 1991, Adams 1999, Vesteggh 1999) mentioning that acids at low concentrations result in the better weight gain and FCR by improving the solubility of the feed ingredients, digestion and absorption of nutrients but higher concentrations were associated with reductions in feed intake and body weights.

HCl secretion is required for protein digestion by activating pepsinogen to pepsin. It also renders the stomach sterile against orally-ingested pathogens, prevents bacterial or fungal overgrowth of the small intestine, encourages the flow of bile and pancreatic enzymes, and facilitates the absorption of a variety of nutrients. It is also known that when the hydrochloric acid production falls short the required amount necessary to maintain the acidity of the white cells and the acid-base balance becomes insufficient and hydrogen chloride eventually vanishes from the circulation. When hydrogen chloride disappears from the circulation some other acid must take its place immediately in order to maintain the pH of the circulating fluids. The acid wastes assume the role of hydrogen chloride in the blood chemistry. This is followed by an imbalance of the blood chemistry.

The HCl medicated chickens also showed significantly reduced oocyst and lesion score. According to several researchers, acids have antimicrobial (Hinton & Linton 1988, Thompson & Hinton 1997, Entani et al. 1998, Chaveerach et al. 2004, Van Immerseel et al. 2004) and antibacterial (Chaveerach et al. 2004, Van Immerseel et al. 2004) activities. However, the anticoccidial activity of the HCl has been reported for the first time.

In the present study, drinking of 1000ppm HCl provided protection against pathogenic effects of *E. tenella* in the intestine probably by declining pH of ceca, obliteration of oocysts and healing of intestinal mucosa in chickens. It is well known fact that the white cells are the essential factor in the healing of wounds, on the chemical side the good

effects come from a glandular and cellular stimulation by the HCl, so making these factors in the production of the acid return to their normal production. However, in using HC1 solution against coccidiosis one must ascertain the correct dosage for even a slightly excessive dose may cause serious intestinal inflammation, with diarrhea and dysentery almost uncontrollable. In the HCl medicated groups (2000ppm and 3000ppm) mortality was also very high and these results are in line with the results as described by Cave (1984) that increasing level of acids cause high mortality.

The pH in the ceca was significantly higher in infected non medicated group than non infected non medicated and all medicated groups. Ruff et al. (1974) suggested that this increase reflects changes in the cecal flora since: 1) the "normal" ceca contain a predominance of acid-producing bacteria (Moore 1969); and 2) the pH in the ceca is significantly greater in germ-free birds than in conventional birds.

A little bit is known regarding the mode of action of acids against bacteria. In this aspect many researchers have different views. According to some researchers, the antibacterial activity of acids is related to the reduction of pH, as well as their ability to dissociate, which is determined by the pKa-value of the respective acid, and the pH of the surrounding milieu. Acids are lipid soluble in the undissociated form, and they easily enter the microbial cell by both passive and carrier-mediated transport mechanisms. Once in the cell, the acids release the proton H^+ in the more alkaline environment, resulting in a decrease of intracellular pH. This influences microbial metabolism, inhibiting the action of important microbial enzymes and forces the bacterial cell to use energy to export the excess of protons H^+, ultimately resulting death by starvation. In the same matter, the protons H^+ can denature bacterial acid sensitive proteins and DNA (Russell & Diez-Gonzalez 1998). Likewise, Hydrocholric acid also ionizes to release H^+ ions.

Data from the present study indicate that lower doses of HCl have anticoccidial activity against *Eimeria tenella* in broiler chickens; therefore, it has the potential to be used as an alternative coccidiosis control agent. With the escalating cost of anticoccidial drugs how nice it would be to replace expensive anticoccidial drugs with dilute hydrochloric acid, the cost of which is nil. But the exact mode of action against *Eimeria* species is not clear. So, further research should be carried out to find out the exact mode of action of HCl against coccidia.

Acknowledgements.- To Prof. Dr. Ahrar Khan, Editor Pakistan Veterinary Journal for his editorial and technical assistance through out the experiment.

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